L2: Entry 10 of 21 File: USPT Feb 18, 2003

DOCUMENT-IDENTIFIER: US 6521407 B1
TITLE: Methods for determining chemosensitivity of cancer cells based upon expression of negative and positive signal transduction factors

Detailed Description Text (35):
Determination of the expression level of Raf-1 is effected by measuring the abundance of the Raf-1 protein. Raf-1 protein levels can be measured by immunocytochemistry or flow cytometry (FCM). Previously there was a problem with the latter approach arising out of cross-reactivity of existing antibodies with non-Raf-1 proteins. Until the present, there were no available antibodies to Raf- which did not also cross-react with a very abundant but irrelevant 48 kD protein on Western blotting. Techniques such as immunocytochemistry or FCM would only give non-specific results. This necessitated some form of molecular separation, such as by electrophoresis in Western blotting, to separate Raf-1 (a 72-74 kD protein) from the irrelevant 48 kD species. Column chromatography techniques were appropriate, such as gel filtration or ion exchange chromatography. High Performance Liquid Chromatography or Capillary Electrophoresis were also usable as separation techniques. These could all be followed by an immunoassay. Other means of specific recognition may also have been conceivable, including the development of RNA aptamers to the Raf-1 protein. However, all of these techniques are time consuming and expensive, making them inappropriate for a clinical test. The availability of an antibody specific to Raf-1 allows diagnostic assays to be carried out without the need for separation.

L2: Entry 12 of 21 File: USPT Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6027892 A
TITLE: Compositions and methods for reducing radiation and drug resistance in cells

Drawing Description Text (3):
FIG. 1. The effect of anti-raf-1 oligonucleotides on Raf-1 p 72 protein synthesis and the radiation resistance levels of SCCHN cell lines JSQ-3 and SQ-20B. A--Western blot analysis of Raf-1 protein synthesis inhibition by increasing concentrations of raf-1 oligonucleotides. C=untreated cells; O=cells treated with liposomes but no oligonucleotides; AS=antisense; S=sense. B--Histogram demonstrating radiosensitization with increasing concentrations (0.1, 0.3, 1 .mu.M) of anti-raf-1 ASO. As controls, the cells were treated with 1 .mu.M of either a sense or a scrambled raf-1 oligonucleotide. Radioresistance levels are given as D.sub.10 values. Error bars represent the standard error of the mean (S.E.M.) of 2 to 13 values.

Drawing Description Text (4):
FIG. 2. Histogram demonstrating the effect of 1 .mu.M anti-raf-1 ASO on SK-OV-3, T24 and MCF10A cells. As controls, the cells were treated with 1 .mu.M of either a sense or a scrambled raf-1 oligonucleotide. Radioresistance levels are given as D.sub.10 values. Error bars represent the S.E.M. of 2-6 values.